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Experimental studies on the reactions of Collembola to copper contamination

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Summary. Laboratory experiments were carried out to explore possible causes for the absence or reduced abundance of certain Collembola species in copper contaminated soils.
The experiments were conducted in plastic boxes filled with undisturbed arable soil. Direct toxicity was tested by spraying the soil surface with a solution containing $CuCl_2 \cdot 3\,Cu(OH)_2$ to achieve a total concentration of 150 µg Cu/g topsoil. After 1 month the mesofauna was extracted from two sets of 12 200 cm^3 soil cores per box. No differences were found in the abundances of the dominant species in treated and untreated soils. *Willemia anophthalma* was found exclusively in treated soils, and the abundance of *Sminthurinus aureus* was significantly higher in untreated cells. The recolonisation of Cu contaminated soils was investigated by inserting cores of defaunated soils from polluted and unpolluted sites into the boxes. They were allowed to recolonise from the surrounding soil for three months. The abundances of *Mesaphorura macrochaeta* and *Folsomia manolachei* were significantly higher in the Cu contaminated soils.
To test the perception of inorganic Cu, the distribution of *Folsomia candida* in Petri dishes was determined. They were kept in the dark and contained cellulose filter or potato pieces that had been soaked in Cu solution or water, respectively. For both types a clear preference for uncontaminated baits was shown.

Key words: Perception, recolonisation, heavy metals, fungicides

Introduction

Long-term application of copper fungicides results in soil contamination. In soils cultivated with hops (*Humulus lupulus* L.) Cu compounds have been applied since 1926, resulting in Cu concentrations of up to 1,000 mg/kg dw (Scheffer & Schachtschabel 1989).
Heavy metal contamination causes a reduction of fungal biomass and retards the process of decomposition due to reduced biotic activity (Bengtsson et al. 1988; Curry 1986; Tyler 1981). In soils with high amounts of organic matter, Collembola as a group are not susceptible to heavy metal contamination (Bengtsson & Rundgren 1988; Hågvar & Abrahamsen 1990; Lübben 1991). However, in highly Cu-contaminated arable soils their abundances are reduced

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(Filser 1993, 1995; Filser et al. 1995) and, in all of the above mentioned studies, species composition differed markedly from unpolluted control sites (Bengtsson & Rundgren 1988; Filser 1992, 1995; Filser et al. 1995; Hågvar & Abrahamsen 1990; Lübben 1991; Tranvik & Eijsackers 1989). Bengtsson et al. (1985) attributed low survival rates in metal polluted soils to reduced fungal biomass rather than to direct toxicity. Growth and survival rates of *Onychiurus armatus* Tullberg 1869 were reduced when fed with Cu/Zn-polluted fungi. Resistance to heavy metals can be either an effect of detoxification mechanisms or of avoidance strategies (Humbert 1974, 1977; Joosse & Verhoef 1983). Many authors have described selective feeding of Collembola on various food sources (Chen et al. 1996; Klironomos & Kendrick 1995; Visser 1985; Walsh & Bolger 1990, and references therein). Heavy metal tolerant fungi have high protein contents which enable them to detoxify metals by complex formation (Bengtsson & Rundgren 1988). Sensitive fungi accumulate heavy metals to a greater extent than tolerant ones (Tranvik & Eijsackers 1989). The abundances of metal tolerant fungi in contaminated soils are important if high numbers of tolerant Collembola are to occur (Bengtsson & Rundgren 1988). Tranvik & Eijsackers (1989) have shown that a resistant collembolan was able to differentiate between heavy metal polluted and unpolluted fungi, whereas this ability was missing in a sensitive species. Besides morphological restrictions, a prerequisite for selective feeding is the ability to distinguish different food sources by olfactory or contact cues. Hedlund et al. (1995) investigated fungal odour discrimination for two sympatric Collembola species. They could explain their reactions by differences in antennal responses to selected organic compounds. To our knowledge, so far no-one has investigated the perception of inorganic compounds by Collembola.

The scope of our study was to elucidate some factors that might explain the presence or absence of Collembola in Cu polluted soils. Our hypotheses were: (i) the application of a Cu fungicide to an unpolluted soil has a negative impact on sensitive species; (ii) sensitive species do not recolonise defaunated contaminated soils and (iii) Collembola are capable of perceiving inorganic Cu compounds.

Materials and Methods

Direct toxicity and recolonisation

The soil was taken from the experimental farm of the FAM (Forschungsverbund Agrarökosysteme München) research network on agroecosystems. It was a sandy colluvium with a pH ($CaCl_2$) of 6.0, 1.1 g C and 0.12 g N per kg dw (more detailed description in Filser et al. 1995). The soil was sampled in late autumn. Each experiment was carried out in a plastic box (40 · 60 cm) filled with six 20 · 20 cm undisturbed soil blocks to a height of 15 cm. They were incubated in the laboratory at an avarage temperature of 13 °C. From time to time they were irrigated with tap water. The surface of each box was divided into 24 squares (10 · 10 cm) to which the treatments were applied at random. Solutions of the fungicide Fitoran were used as the Cu containing compound. The chemical formula is $CuCl_2 \cdot 3\ Cu\ (OH)_2$ which amounts to 45 % Cu. Samples of the soil fauna were taken from the upper 4 cm with a cylindrical corer (diameter: 7.8 cm) and extracted with a modified Macfadyen extractor over six days. Ethylene glycol served as collection fluid. The Collembola were mounted on slides and identified to species according to Dunger & Zivadinovic (1989), Fjellberg (1980), Gisin (1960), and Palissa (1964).

For direct toxicity testing, 20 ml of a 15 % Cu solution was applied to half of the squares, the remainder were sprayed with the same amount of tap water. The treatments resulted in 151 mg Cu/kg dw in the upper 4 cm, the untreated plots contained 15 mg Cu kg dw. One month after Cu application the mesofauna were extracted.

The recolonisation experiment followed a design of Hågvar & Abrahamsen (1980). Sixteen cylindrical soil cores (7.8 · 4 cm) were taken from the boxes. Half of them were discarded, the rest replaced in nylon mesh baskets (mesh width: 1.25 mm) after defaunation by deep-freezing (–75 °C, 20 h). The discarded cores were replaced by cores from contaminated soil that had been treated in the same way. The contaminated soil originated from a field next to the one of the control soil with similar texture. It had been cropped with hops for a long time which resulted in a Cu contamination of 200 mg/kg dry soil. The pH ($CaCl_2$) was 6.1, the C and N contents 1.6 and 0.16 mg/kg dw. Mesofaunal recolonisation of the cylinders was allowed for 16 weeks after which the mesofauna were extracted.

Perception of Cu

The experimental setup follows Strebel (1932). For each experiment three Petri dishes (diameter: 9 cm) were filled with a plaster of Paris /charcoal (3 : 1) mixture and subdivided into 12 equal segments. At the edge of each sector, round cellulose filter pieces (diameter: 5 mm) were placed and provided with 100 µl of water, 15 % or 30 % Cu solution, corresponding to 0; 1,500; 3,000 mg Cu/kg dw. They were placed in a consecutive order to maintain a gradient within the dish. Ten individuals of *Folsomia candida* (Willem 1902) were placed in the middle of the dish with the aid of a plastic ring. The dishes were kept in the dark at room temperature for two days. A photograph was taken every 30 minutes during 3 hours of the two days (n = 39). For evaluation the animals in each sector were counted and attributed to one of the concentrations. In the second experiment boiled potatoes were soaked over night in the test solutions to provide a food source. A total of 6 pieces (0.5 cm^3) was added to each dish in the same manner. The animals were hard to recognise since they were often in the potato pieces. Therefore, the number of records was increased to a total of n = 61 over three days.

Results

The reactions of the Collembola to direct Cu treatment differed between species (Fig. 1). Some species were not affected at all. *Willemia anophthalma* Börner 1901 was more abundant in the treated plots, *Sminthurinus aureus* (Lubock 1862) in the controls. Due to low numbers of individuals, no further significant differences were found.
In the recolonisation experiment *Isotomurus palustris* (Müller 1776) and *Onychiurus armatus* established very high population densities (corresponding to about 500,000 individuals per m^2), but abundances of neither differed between polluted and uncontaminated soil (Fig. 2). The only significant differences were found for *Mesaphorura macrochaeta* (Rusek 1976) and *Folsomia manolachei* Bagnall 1931 (sensu Deharveng 1982) which were both more abundant in the contaminated soil.
Folsomia candida was able to differentiate Cu contaminated from uncontaminated areas (Fig. 3). The zero and medium concentration sectors were clearly preferred, only 18 % of the animals were found in the high concentration sectors. The differences between the single concentrations became more obvious when potato pieces were offered: more than 50 % of the animals were counted in sectors containing uncontaminated pieces.

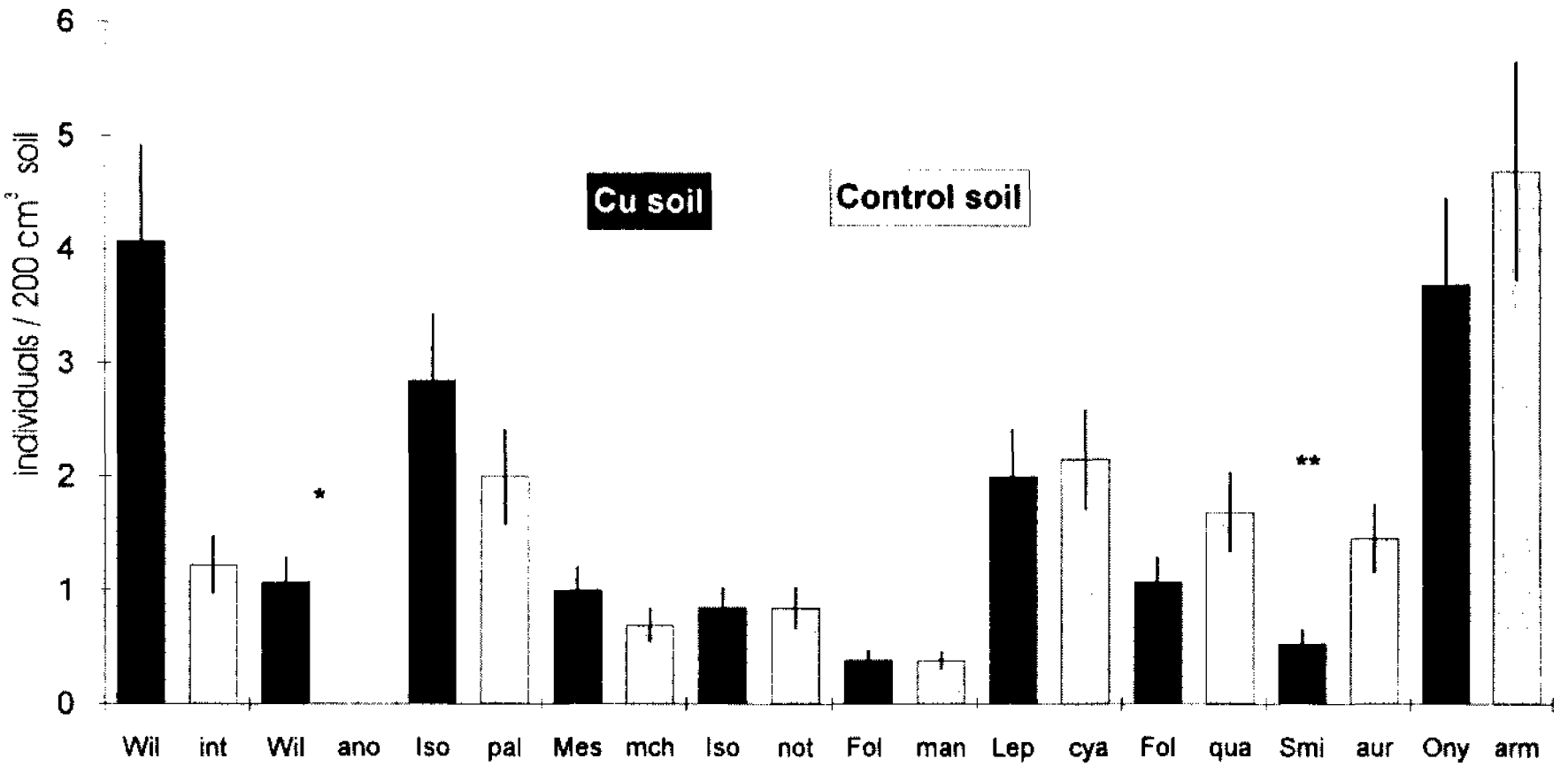

Fig. 1. Collembola abundances in soils treated with Cu solution and controls (means ± SE, n = 12). Significant differences (Mann-Whitney U test) are indicated by asterisks * $p < 0.05$, ** $p < 0.01$. Wil int = *Willemia intermedia*, Wil ano = *Willemia anophthalma*, Iso pal = *Isotomurus palustris*, Mes mch = *Mesaphorura macrochaeta*, Iso not = *Isotoma notabilis*, Fol man = *Folsomia manolachei*, Lep cya = *Lepidocyrtus cyaneus*, Fol qua = *Folsomia quadrioculata*, Smi aur = *Sminthurinus aureus*, Ony arm = *Onychiurus armatus*

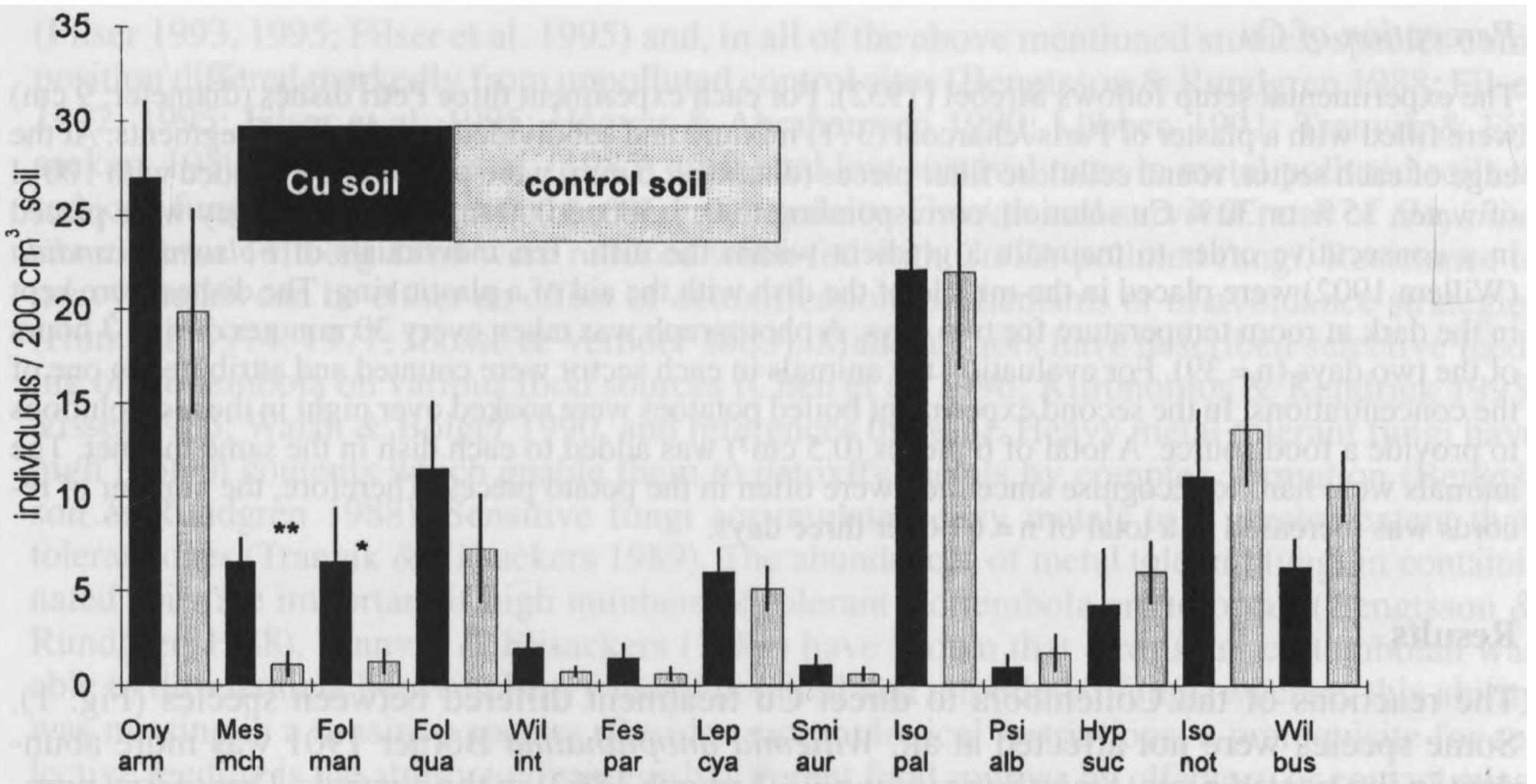

Fig. 2. Collembola abundances in defaunated Cu and control soils after 16 weeks of recolonisation from control soil (means ± E, n = 8). Significant differences (Mann-Whitney U test) are indicated by asterisks * $p < 0.05$, ** $p < 0.01$. Fes par = *Folsomides parvulus*, Psi alb = *Pseudosinella alba*, Hyp suc = *Hypogastrura succinea*, Wil bus = *Willowsia buski*. Other species abbreviations see Fig. 1

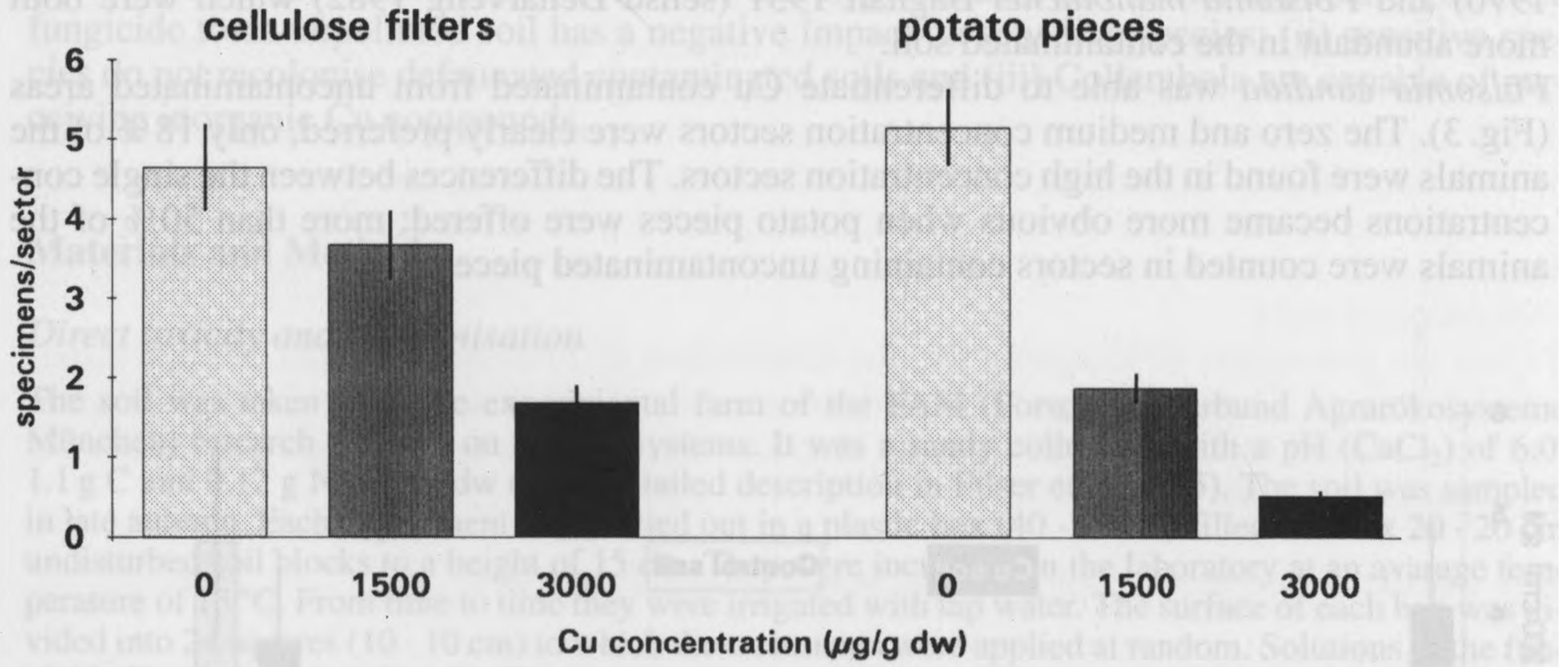

Fig. 3. Effects of different Cu concentrations on the distribution of 10 *Folsomia candida* in petri dishes. (means ± SE; application to cellulose filters: n = 39; application to potato pieces: n = 61). For both applications the distributions are statistically significant ($p < 0.001$, Friedmann's Two-Way Anova)

Discussion

The numbers of individuals in the direct toxicity experiment were generally very low, probably as a result of the extremely wet outdoor conditions just before the experimental soil was taken. Therefore, the results have to be interpreted with care. *S. aureus*, *Willemia intermedia* Mills 1934 and *W. anophthalma* occur only in low abundances in hops (Filser 1992) and are negatively influenced by sewage sludge treatments (Lübben 1991). The application of high amounts of Cu fungicides to the soil surface had a significant negative impact on the first species, whereas both *Willemia* species showed the opposite reaction. Bengtsson & Rundgren

(1988) found no distinct heavy metal effects on the distribution of *W. anophthalma*. One has to consider that a single application of extremely high amounts of Cu does not reflect agricultural practice. Due to the very low mobility of Cu (Wittassek 1987) the main proportion will remain at the surface, whereas in arable land it is also found deeper in the soil due to tillage. Euedaphic species thus probably had no contact with the copper which could partly explain the higher abundances of both *Willemia* species and *M. macrochaeta* in the treated plots. They may have had better food access as *O. armatus* (also euedaphic) tended to be more abundant in untreated plots. In experiments carried out by Bengtsson et al. (1994) the latter species avoided contaminated areas. Abundances of none of the other species differed significantly between treated and untreated plots. We conclude that the characteristic species composition of polluted soils may be attributed to long-term adaptation of fauna and microflora to constant heavy metal enrichment rather than to direct toxicity.

The results of the direct toxicity and recolonisation experiments cannot be compared directly. Both vertical distribution and quality of Cu differed due to the manner of contamination (short-term application of high doses vs. accumulation of moderate doses over a very long time). Thus, the Cu soil from the field probably contained a microflora adapted to Cu contamination. The recolonisation experiment confirmed the success of *M. macrochaeta* in metal polluted soils described by various authors (Filser et al. 1995; Lübben 1991; Bengtsson & Rundgren 1988). The high abundances of *F. quadrioculata* (Tullberg 1871) and *F. manolachei* in the polluted soils contradict all field data as in hop soils both species are completely lacking (Filser 1993), and also in forest soils the abundance of *F. quadrioculata* decreases with increasing heavy metal concentration (Bengtsson & Rundgren 1988). Hågvar & Abrahamsen (1990) found an increase of *F. quadrioculata* in highly lead-contaminated soils which shows that heavy metal effects must not be generalised. Our data suggest that both *Folsomia* species may have colonised the contaminated soils due to their higher organic matter content which usually provides better microclimatic and food conditions. It has been shown previously that some Collembola have the best growth and reproduction rates when feeding on other than the preferred fungal species (Shaw 1988, cited from Walsh & Bolger 1990). It is probable that the *Folsomia* species are not capable of discriminating between Cu tolerant and sensitive fungi, as has been described for *Isotomiella minor* (Tranvick & Eijsackers 1989). Thus, they may feed on sensitive – i.e. Cu accumulating – fungi which, in the long run, may lead to reduced abundance or even extinction. However, this could not be proven in a 16-week experiment.

Cu compounds either drip directly to arable soils during fungicide applications, or they are deposited with crop residues which can contain up to 1,700 µg Cu per g dw (Filser 1993). Thus, soil organisms are confronted with a highly-contaminated food source. Therefore it might be important to perceive inorganic as well as organic Cu. Our data clearly show that *Folsomia candida* is capable of discriminating between contaminated and uncontaminated areas. In sewage-sludge treated arable fields this species was significantly more abundant than in untreated fields (Lübben 1991), and Bengtsson & Rundgren (1988) found its highest abundances at moderately polluted sites. These findings hint at an advantage over other species due to the perception and avoidance of contaminated microsites. Consequently, the success of heavy metal tolerant Collembola might not only be attributed to detoxification by formation and subsequent excretion of mineral concretions in the midgut epithelium (Humbert 1974, 1977; Joosse & Verhoef 1983) and fungal odour discrimination (Hedlund et al. 1995; Tranvick & Eijsackers 1989) but also to inorganic Cu perception.

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